PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 May 2001 (02.05.01)	
International application No. PCT/US00/19239	**Applicant's or agent's file reference** 0974/2F828-W
International filing date (day/month/year) 14 July 2000 (14.07.00)	**Priority date** (day/month/year) 14 July 1999 (14.07.99)
Applicant JESTY, Jolyon et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 February 2001 (12.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
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PA T COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 0974/2F828-W	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/19239	International filing date *(day/month/year)* 14 July 2000 (14.07.2000)	Priority date *(day/month/year)* 14 July 1999 (14.07.1999)

International Patent Classification (IPC) or national classification and IPC

IPC(7): C12N 7/04, 9/74; C12Q 1/00, 1/02, 1/56; G01N 33/53, 33/567; A61K 38/48 and US Cl.: 422/50, 55, 63, 65, 68.1, 81, 82, 82.09, 93, 400; 435/286.5, 286.6, 287.1, 287.3, 287.6, 288.4; 436/52, 53, 54, 165, 172, 180

Applicant

THE RESEARCH FOUNDATION OF STATE UNIV. OF NEW YORK

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12 February 2001 (12.02.2001)	13 September 2001 (13.09.2001)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence for Gailene R. Gabel Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International appl[redacted] No.
PCT/US00/19239

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-23 as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

☒ the claims:
pages NONE , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages 24-26 , filed with the letter of 13 AUGUST 2001.

☒ the drawings:
pages 1-4 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	6,7,11,12 and 16-18	YES
	Claims	1-5,8-10,13-15,19 and 20	NO
Inventive Step (IS)	Claims	6,7,11,12 and 16-18	YES
	Claims	1-5,8-10,13-15,19 and 20	NO
Industrial Applicability (IA)	Claims	1-20	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Please See Continuation Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

V. 2. Citations and Explanations:

Claims 1-5 and 8-10 lack novelty under PCT Article 33(2) as being anticipated by Zou et al. (US 5,677,162).

Zou et al. disclose catalyzing modified prothrombinase substrate in the presence of factor X , factor V, and phospholipid vesicle into modified thrombin (see column 2). Prothrombin complex composition is modified (isolated) by suspension into polyethylene glycol (PEG). The time for activation of prothrombin to thrombin is determined by measuring thrombin activity at various stages and by conversion of fibrinogen into fibrin (see column 3).

Claims 1-5 and 8-10 lack novelty under PCT Article 33(2) as being anticipated by Kaetsu et al. 1 (US 5,811,279).

Kaetsu et al. 1 disclose catalyzing the conversion of modified protrhrombinase substrate into modified thrombin in the presence of Factor X. Specifically. Kaetsu et al. 1 teach that prothrombin is modified or treated with the addition of PEG (see column 4). The thrombin formed exhibits activation rate (see Example 1).

Claims 1-5 and 8-10 lack novelty under PCT Article 33(2) as being anticipated by Kaetsu et al. 2 (Thrombosis Research, Feb 1998).

Kaetsu et al. 2 teach accelerating catalysis of prothrombin modified with PEG to generate thrombin in a mixture of prothrombin and factor X (see Abstract). Kaetsu et al. 2 specifically found that PEG potentiates Factor Xa-mediated activation of prothrombin (see Abstract).

Claims 13-15 and 19-20 lack an inventive step under PCT Article 33(3) as being obvious over Zou et al. (US 5,677,162), Kaetsu et al. (US 5,811,279), Kaetsu et al. (Thrombosis Research, Feb 1998).

Zou et al., Kaetsu et al. 1, and Kaetsu et al. 2 have been discussed supra.

Zou et al., Kaetsu et al. 1, and Kaetsu et al. 2 fail to teach kit formats for use with the claimed method.

It would have been obvious to one of ordinary skill in the art at the time of the instant to incorporate the reagents used by Zou, Kaetsu 1, and Kaetsu 2 into a kit format because kit formation is well within ordinary skill, conventional, and well-known for its advantage in convenience and economy.

Claims 6-7, 11-12, and 16-18 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest prothrombinase substrate chemically derivatized by the addition of acetyl group donated by sulfo-N-succinimidyl acetate or as product of an allele of prothrombin gene selected from Metz and Quick I.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) in the field of hematology and coagulation studies.

Response to Arguments

A) Applicant submit that the claimed method, as amended, is not anticipated by all cited references including Zhou et al., Kaetsu et

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

al.-1, and Kaetsu et al.-2 because each cited reference fails to teach a method comprising converting a modified prothrombinase substrate (prothrombin) to a modified prothrombinase product (thrombin) which does not activate platelet.

In response, Applicant's definition of "modified thrombin" in the specification does not appear to exclude, or otherwise encompasses, some level of platelet activating activity by the thrombin (see page 7, lines 10-15). Therefore, Applicant's disclosure fails to substantially support the amended claim as currently recited. Accordingly, the teaching of all cited references read on the claims as amended.

B) Applicant argues that the mere presence of PEG or acetate or Ca in no way teaches or suggests that the PEG or acetate in any way modifies, i.e. binds or conjugated to, any molecule dissolved in the substrate.

In response, all cited references including Zhou et l., Kaetsu et al.-1, and Kaetsu et al.-2 appear to disclose treatment of the sample with PEG, or otherwise, converting prothrombin to thrombin in the presence of PEG, in order to potentiate enzyme activation of prothrombin and does not appear to exclude chemically modifying the prothrombin or thrombin. Absence evidence to the contrary, the PEG present in the methods of Zhou, Kaetsu-1, and Kaetsu-2 would have been able to inherently chemically modify the prothrombin and thrombin present the sample.

C) Applicant argues that the claimed kits are not obvious over Zhou et al., Kaetsu et al.-1, and Kaetsu et al.-2 because none of the cited references suggest each of the claimed components.

In response, it would have been obvious to one of ordinary skill in the art at the time of the instant to incorporate the reagents and components used by Zou, Kaetsu 1, and Kaetsu 2 into a kit format because kit formation is well within ordinary skill, conventional, and well-known for its advantage in convenience and economy.

---------- NEW CITATIONS ----------

WHAT IS CLAIMED IS:

1. A method for assaying an activation state of a platelet comprising detecting catalysis of a modified prothrombinase substrate to a modified prothrombinase product, wherein said product does not activate platelets, by a prothrombinase which is associated with the platelet.

2. The method of claim 1 wherein the detection of the catalysis of a modified prothrombinase substrate comprises detecting the production of modified thrombin , wherein said thrombin does not activate platelets.

3. The method of claim 1 wherein detecting the catalysis of a modified prothrombinase substrate comprises detection of modified thrombin catalytic activity.

4. The method of claim 1 wherein the prothrombinase enzyme comprises factor Xa, factor Va and one or more members selected from the group consisting of a PS:PC vesicle and a platelet.

5. The method of claim 1 wherein the modified prothrombinase substrate comprises prothrombin which is chemically derivatized by the addition of one or more chemical groups selected from the group consisting of an acyl group, an acetyl group, a succinyl group, a maleyl group, a polyethylene glycol group, an acetylated polyethylene glycol group, a pyridoxal 5'-phosphate group and a dichlorotriazinylaminofluorescinyl group.

6. The method of claim 5 wherein the modified prothrombinase substrate comprises prothrombin which is chemically derivatized by the addition of an acetyl group wherein the acetyl group is donated by sulfo-N-succinimidyl acetate.

7. The method of claim 1 wherein the modified prothrombinase substrate is a

product of an allele of a prothrombin gene selected from the group consisting of *Metz* and *Quick I*.

8. The method of claim 2 wherein the detection of modified thrombin comprises an assay selected from the group consisting of a Western blot, an Enzyme Linked ImmunoSorbent Assay, an immunodiffusion assay, a surface plasmin resonance assay, and a fluorescence proximity assay.

9. The method of claim 3 wherein the detection of modified thrombin catalytic activity comprises detecting fibrin.

10. The method of claim 3 wherein the detection of modified thrombin catalytic activity comprises detecting fibrinogen.

11. The method of claim 3 wherein the detection of modified thrombin catalytic activity comprises detecting cleavage of a peptide.

12. The method of claim 11 wherein the peptide is glycyl-L-prolyl L-arginine wherein the amino terminal end of the peptide is linked to a tosyl group and the carboxyl terminal end of the peptide is linked to a p-nitroanalide group.

13. A kit for assaying an activation state of a platelet comprising:

(a) a prothrombinase substrate which has been modified so that, when catalyzed by prothrombinase, a modified prothrombinase product which does not activate platelets is produced; and

(b) a prothrombinase product assay.

14. The kit according to claim 13 wherein the prothrombinase product assay is selected from the group consisting of a Western blot, an Enzyme Linked ImmunoSorbent Assay (ELISA), an immunodiffusion assay, a surface plasmin resonance assay, a chromogenic peptide cleavage assay, a polyacrylamide gel electrophoresis analysis, and a

fluorescence proximity assay.

15. The kit of claim 13 wherein the modified prothrombinase substrate is prothrombin which is chemically derivatized by the addition of one or more chemical groups selected from the group consisting of an acyl group, an acetyl group, a succinyl group, a maleyl group, a polyethylene glycol group, an acetylated polyethylene glycol group, a pyridoxal 5'-phosphate group and a dichlorotriazinylaminofluorescinyl group.

16. The kit of claim 13 wherein the modified prothrombinase substrate is a product of an allele of a prothrombin gene selected from the group consisting of *Metz* and *Quick I.*.

17. The kit of claim 13 wherein the prothrombinase product assay comprises reagents for a chromogenic peptide cleavage assay wherein the reagents comprise a peptide having a sequence cleaved by thrombin.

18. The kit of claim 17 wherein the peptide is glycyl-L-prolyl L-arginine wherein the amino terminal end of the peptide is crosslinked to a tosyl group and the carboxyl terminal end of the peptide is crosslinked to a p-nitroanalide group.

19. The kit of claim 13 further comprising one or more reagents selected from the group consisting of human α-thrombin, calcium ionophore A23187, factor Xa, Sulfo-N-succinimidyl acetate, factor Va and phospholipid vesicles comprising phosphatidylserine and phosphatidylcholine.

20. The kit of claim 13 further comprising one or more components selected from the group consisting of a glass vial, a microtiter plate, water and a syringe.